

Comparative Karyology of Lentic and Lotic Populations of *Diplodon chilensis chilensis* (Bivalvia: Hyriidae)

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Abstract. The mean karyotypes of four populations of *Diplodon chilensis chilensis* (Gray, 1828) (two lentic and two lotic) inhabiting the south of Chile are described and compared. Chromosomes were obtained by squash of cleaving embryos, previously treated with colchicine, fixed in ethanol-acetic acid 3:1 at 4°C, and stained using the Feulgen reaction. The karyotype of the four populations is $2n = 34$, with metacentric and submetacentric chromosomes. In the population from the Peu-Peu stream, the centromeric position in two chromosome pairs (3 and 10) differs significantly from that in the corresponding pairs from the other populations. Karyotype findings of this study do not support the proposal that the differences described between lentic and lotic populations of *D. ch. chilensis* may result from genetic differentiation among populations. This study is an initial contribution to the knowledge of cytogenetic characteristics in Chilean populations of *Diplodon chilensis chilensis*.

INTRODUCTION

Diplodon chilensis chilensis (Gray, 1828) is a freshwater bivalve widely distributed in lakes and rivers in central and southern Chile, including Chilean and Argentinian Patagonia (Peredo & Parada, 1986). In recent years, changes have occurred in the waters inhabited by populations of this species, accompanied by serious pollution by a number of biological and chemical agents. As a result, many of these populations, especially in the rivers, have been depleted.

It is generally accepted that the preservation of biodiversity requires the maintenance of genetic diversity within species, especially those which are endangered (Berg et al., 1998). This in turn supposes the need for contributions of up-to-date information on the reproductive, ecological, and genetic characteristics of the species, which together may be used to develop conservation strategies with the aim of preserving the genetic diversity of *D. ch. chilensis*.

The reproductive and ecological characteristics of *Diplodon chilensis* have been extensively described and discussed by Parada et al. (1989a, b and 1990), and Parada & Peredo (1994). These studies demonstrate that individuals of lentic and lotic populations of *D. ch. chilensis* vary widely between populations, both in phenotype and in their life strategies.

Parada & Peredo (1994) proposed the hypothesis that the differences described between river and lake popu-

lations correspond to different patterns of phenotype plasticity as an adaptive response to the particular environmental conditions of the population's habitats. In this study we suggest the possibility that these differences may result from genetic differentiation among the populations, with phenotype plasticity superimposed. This genetic differentiation, depending on its magnitude, may involve mechanisms of chromosome evolution (rearrangement, duplication) which could have generated significant changes in the karyotype morphology of some populations. There are no previous genetic studies whose results would serve to either confirm or refute this prediction.

The aim of this study is to describe and compare the karyotypes of two lentic populations located in different hydrographic systems (Lake Villarrica, which belongs to the River Tolten basin, and Lake Panguipulli, belonging to the River Valdivia basin) and two lotic populations in streams (Peu-Peu and Botrollhue) which are tributaries of the River Cautin, belonging to the River Imperial basin.

MATERIALS AND METHODS

One hundred adult specimens from each population of *Diplodon chilensis chilensis* were used, taken from two lentic environments: Lake Villarrica (39°18'S, 72°05'W) and Lake Panguipulli (39°43'S, 72°13'W) and two lotic: Botrollhue stream (38°43'S, 72°45'W) and Peu-Peu stream (38°31'S, 72°22'W) all belonging to hydrographic systems in the south of Chile (Figure 1). The description of the karyotype of the Lake Villarrica population was taken from Jara-Seguel et al. (2000).

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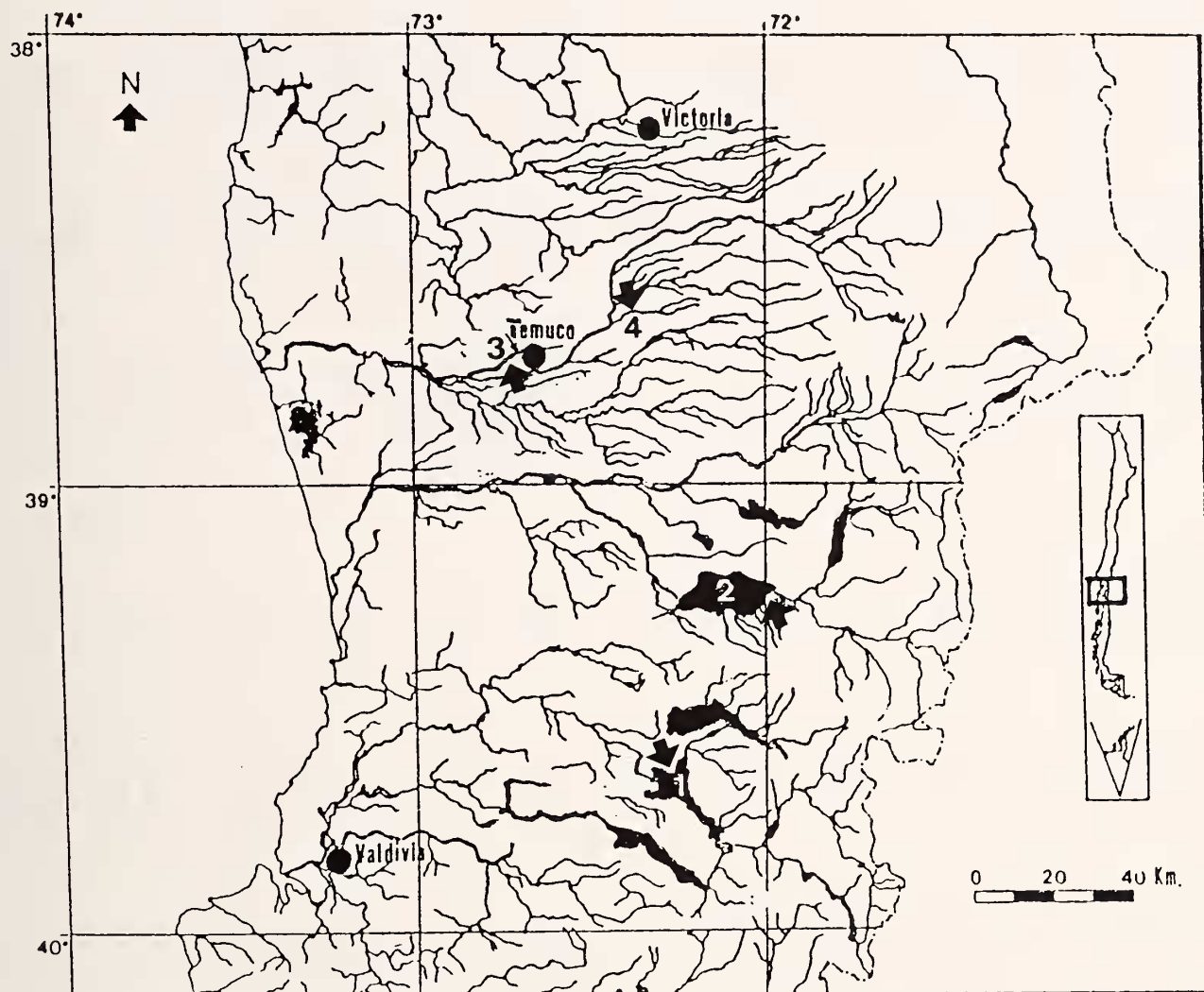


Figure 1. Geographic location of the areas inhabited by the populations of *D. ch. chilensis* studied. 1. Lake Panguipulli. 2. Lake Villarrica. 3. Botrolhue stream. 4. Peu-Peu stream.

In the laboratory, the specimens were sexed, and cleaving embryos were removed from the inner demibranchs of gravid females. The embryos were treated with colchicine at 0.05% for 3 hours, fixed in ethanol-acetic acid 3:1 at 4°C for 24 hours, and stained using the Feulgen reaction. The chromosomes were obtained by squash. For each population, 60 metaphase plates were counted, using embryos extracted from 10 to 15 females, and the 10 plates with clearest results were photographed. In photographs, the chromosomes were measured and the relative length was determined (expressed as a percentage of the total haploid set length). The absolute lengths (in μm) of the short arm (SA) and long arm (LA) were established for each chromosome pair. The values for mean relative length for SA and LA for each population (\pm confidence interval at 95%) were displayed in a karyo-idiogram (Palma-Rojas et al., 1997). Ten karyotypes were prepared for

each population. The mean absolute chromosome size values were compared using ANOVA and L.S.D. tests at 95% confidence.

RESULTS

The four populations of *D. ch. chilensis* studied show a diploid karyotype of $2n = 34$ (Figures 2a–d).

The karyo-idiogram (Figure 3), shows that the karyotype for the Lake Villarrica, Lake Panguipulli, and Botrolhue stream populations is composed of nine pairs of chromosomes located in the metacentric area (m) and eight pairs located in the submetacentric area (sm). On superimposing the confidence intervals for the relative lengths of the short arm and long arm for the equivalent chromosome pairs in these populations, it can be seen that there are no significant differences in the centromeric po-

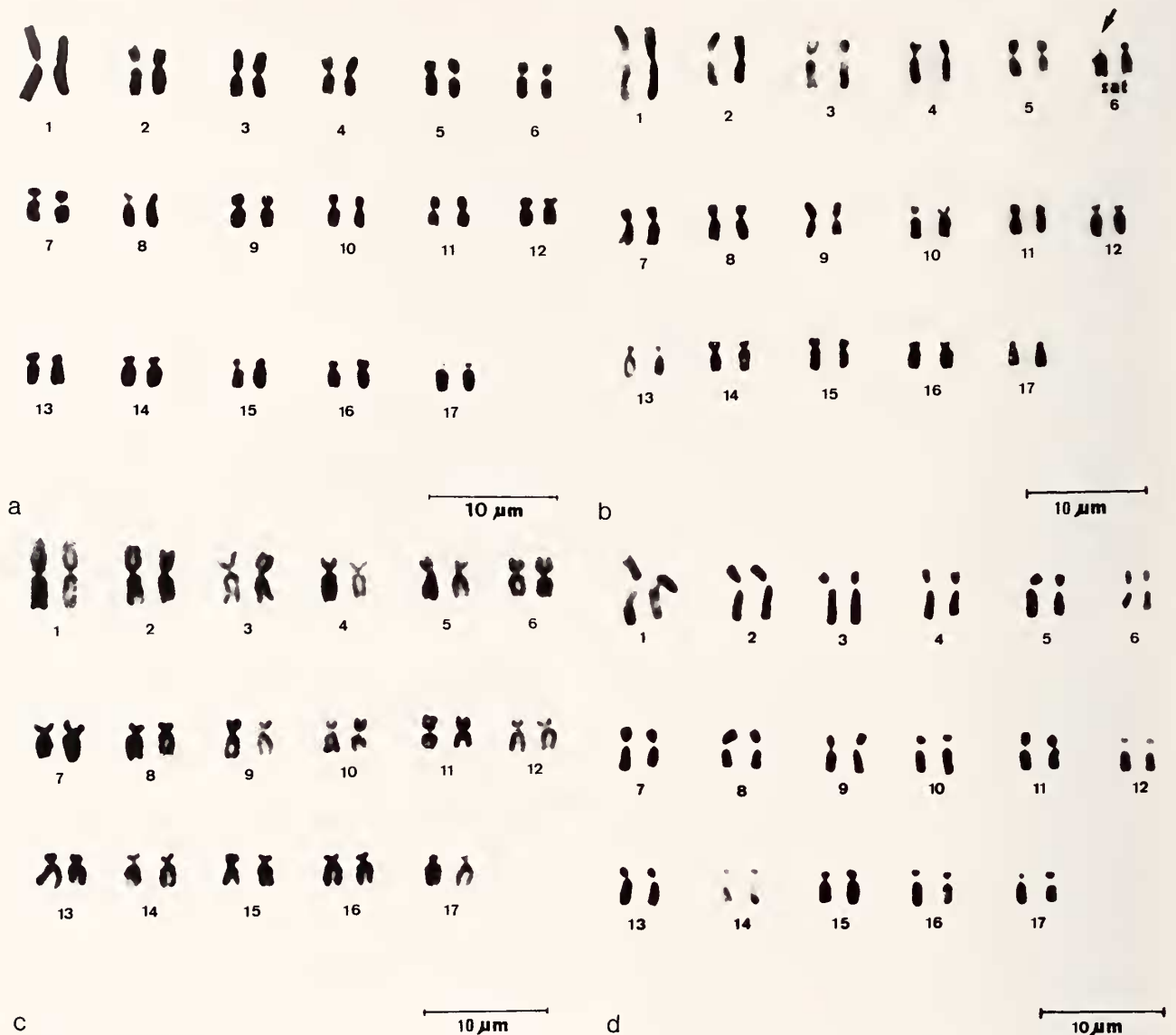


Figure 2. Karyotypes of *Diplodon chilensis chilensis* lentic and lotic populations. a. Lake Villarrica population. b. Lake Panguipulli population. Arrow shows secondary constriction. c. Botrollhue stream population. d. Peu-Peu stream population.

sition ($P > 0.05$), which would therefore indicate that these three populations share a similar karyotype morphology.

The population from the Peu-Peu stream shows two pairs of chromosomes (3 and 10) in which the centromeric position differs significantly from that in the corresponding pairs from the other three populations. Pairs 5 (sm), 7 (m), 8 (m), and 9 (sm), when their confidence intervals are superimposed, show no significant differences in their chromosome morphology when compared with the corresponding pairs in the other populations.

An important cytogenetic factor in this study was the observation in the Lake Panguipulli population of a sec-

ondary subtelomeric constriction in the short arm of chromosome pair number 6 (Figure 2b). This secondary constriction is probably the carrier of an active nucleolar organizer region (NOR). This chromosome pair is also characterized by the fact that it is heteromorphic, since the secondary constriction is only apparent in one of the chromosomes of the pair. This characteristic was observed in 60% of the total metaphasic plates analyzed.

The relative and absolute size of each chromosome pair for the karyotype of each of the four populations is shown in Table 1. The first two chromosome pairs in each population, whose relative length is greater than 8%, are twice the length of the small chromosomes in pairs 16

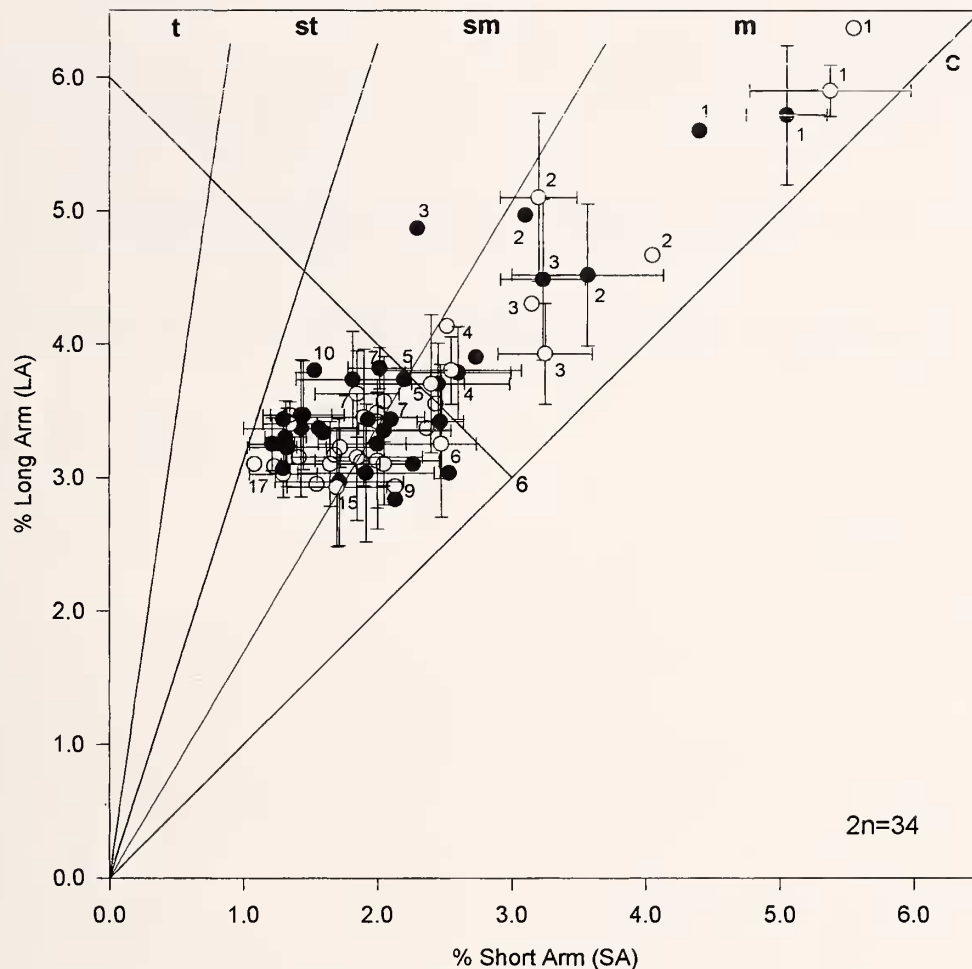


Figure 3. Karyo-idiogram of *Diplodon chilensis* lentic and lotic populations. t = telocentric; st = subtelocentric; sm = submetacentric; m = metacentric LA = long arm; SA = short arm; C = chromosome size percentage. Vertical and horizontal bars represent 95% confidence interval. ● = Lake Panguipulli and Lake Villarrica populations; ○ = Peu-Peu and Botrolhue stream population.

and 17, whose relative lengths do not exceed 5% (Figure 2).

DISCUSSION

The characteristics of the karyotype morphology observed in the Villarrica (Jara-Seguel et al., 2000), Panguipulli, Botrolhue, and Peu-Peu populations, based on the terminology established by Stebbins (1971), allow us to classify the karyotype of *Diplodon chilensis chilensis* as moderately symmetrical, since, although it is composed of metacentric and submetacentric chromosomes, there is a marked difference in the relative size of the chromosomes (Table 1).

The only known information on the chromosome numbers of phylogenetically related species to *Diplodon* are for populations of *Unio tumidus* Philipsson, 1788, and *U. pictorum* (Linnaeus, 1758) (Unionidae) reported by Barsienè (1994). These species show a diploid karyotype of

$2n = 38$ with metacentric and submetacentric chromosomes.

The heteromorphism described between the chromosomes of the pair number 6 in the Lake Panguipulli population may be explained by a differentiating transcriptional function of the ribosomal genes, similar to that described in the karyotype of *Tiostrea chilensis* (Ladrón de Guevara et al., 1996). The use of silver staining techniques will make it possible to identify unequivocally the location of active ribosomal genes within the karyotype.

When the average absolute chromosome size for the Villarrica, Botrolhue, and Panguipulli populations is compared, very similar values are observed, which do not differ significantly from one population to another ($P > 0.05$). However, the chromosome size in the Peu-Peu population is significantly greater ($P < 0.05$) as compared to the other three populations (Table 2). These differences suggest a probable increase in the nuclear DNA content

Table 1

Relative mean length in μm (\pm confidence interval (CI) at 95%) of the short arm (SA) and long arm (LA) of each chromosome pair, chromosome shape (SH) and chromosome size (ChS) of *D. ch. chilensis* populations studied. N = 10 for each population.

Chro- mos. pair	Lake Villarrica				Lake Panguipulli				Botrolhue Stream				Peu-Peu Stream			
	SA (CI)	LA (CI)	SH	ChS	SA (CI)	LA (CI)	SH	ChS	SA (CI)	LA (CI)	SH	ChS	SA (CI)	LA (CI)	SH	ChS
1	5.37 (0.60)	5.90 (0.19)	m	6.5	5.55 (0.39)	6.36 (1.02)	m	6.3	5.05 (0.30)	5.71 (0.52)	m	6.9	4.40 (0.43)	5.61 (0.57)	m	6.6
2	3.20 (0.28)	5.10 (0.63)	m	4.8	4.05 (0.91)	4.66 (0.75)	m	4.8	3.56 (0.56)	4.51 (0.53)	m	5.0	3.12 (0.58)	4.90 (0.63)	m	5.3
3	3.25 (0.35)	3.92 (0.37)	m	4.1	3.15 (0.34)	4.3 (0.53)	m	4.4	3.23 (0.31)	4.48 (0.6)	m	4.3	2.31 (0.33)	5.11 (0.50)	sm	4.9
4	2.55 (0.52)	3.80 (0.25)	m	3.7	2.51 (0.21)	4.13 (0.14)	m	3.7	2.60 (0.39)	3.78 (0.34)	m	3.9	2.51 (0.37)	3.91 (0.24)	m	4.3
5	2.40 (0.58)	3.70 (0.51)	m	3.5	2.43 (0.24)	3.55 (0.34)	m	3.6	2.45 (0.19)	3.70 (0.30)	m	3.5	1.91 (0.33)	3.72 (0.38)	sm	3.8
6	2.47 (0.26)	3.25 (0.54)	m	3.3	2.36 (0.27)	3.36 (0.41)	m-sat	3.4	2.46 (0.17)	3.41 (0.42)	m	3.3	2.51 (0.23)	3.00 (0.45)	m	3.7
7	1.85 (0.31)	3.62 (0.32)	sm	3.2	2.05 (0.12)	3.56 (0.24)	sm	3.4	2.01 (0.23)	3.81 (0.15)	sm	3.2	2.10 (0.22)	3.41 (0.23)	m	3.7
8	1.90 (0.45)	3.45 (0.51)	sm	3.1	2.00 (0.47)	3.48 (0.52)	sm	3.2	1.81 (0.42)	3.73 (0.36)	sm	3.2	2.40 (0.44)	3.10 (0.46)	m	3.6
9	2.00 (0.46)	3.12 (0.51)	m	3.0	2.00 (0.50)	3.41 (0.46)	m	3.2	2.05 (0.50)	3.35 (0.55)	m	3.1	2.03 (0.48)	3.41 (0.50)	sm	3.6
10	2.05 (0.20)	3.10 (0.16)	m	3.0	2.13 (0.33)	2.93 (0.47)	m	3.0	2.00 (0.44)	3.25 (0.48)	m	2.9	1.51 (0.32)	3.80 (0.37)	sm	3.6
11	1.85 (0.48)	3.15 (0.47)	m-sm	2.9	1.88 (0.46)	3.11 (0.49)	m	2.9	1.91 (0.50)	3.03 (0.51)	m	2.9	2.21 (0.48)	2.82 (0.49)	m	3.3
12	1.72 (0.32)	3.22 (0.14)	sm	2.9	1.68 (0.27)	3.16 (0.46)	sm	2.8	1.45 (0.30)	3.46 (0.40)	sm	2.8	1.51 (0.29)	3.42 (0.33)	sm	3.3
13	1.65 (0.36)	3.10 (0.31)	sm	2.8	1.35 (0.11)	3.46 (0.27)	sm	2.8	1.43 (0.42)	3.46 (0.31)	sm	2.8	1.51 (0.29)	3.13 (0.29)	sm	3.3
14	1.35 (0.20)	3.40 (0.16)	sm	2.8	1.41 (0.12)	3.15 (0.33)	sm	2.8	1.43 (0.42)	3.36 (0.51)	sm	2.6	1.51 (0.24)	3.31 (0.33)	sm	3.3
15	1.7 (0.37)	2.92 (0.44)	sm	2.6	1.55 (0.39)	2.95 (0.44)	sm	2.7	1.71 (0.47)	2.96 (0.47)	sm	2.6	1.31 (0.41)	3.32 (0.45)	sm	3.2
16	1.34 (0.29)	3.20 (0.29)	sm	2.6	1.23 (0.12)	3.08 (0.20)	sm	2.7	1.31 (0.15)	3.30 (0.27)	sm	2.5	1.42 (0.18)	3.22 (0.25)	sm	3.2
17	1.25 (0.25)	3.07 (0.17)	sm	2.5	1.08 (0.24)	3.10 (0.36)	sm	2.6	1.21 (0.16)	3.25 (0.19)	sm	2.3	1.32 (0.21)	3.02 (0.24)	sm	2.9

Table 2

Total haploid set length and chromosome mean size (\pm standard deviation) for the populations of *Diplodon chilensis chilensis* studied. Mean karyotype values of *Unio tumidus* from Barsienè (1994). Length and sizes in μm .

Populations	Total haploid set length	Chromosome mean size
Lentic populations:		
Lake Villarrica	57.2	$3.37 \pm 0.99^*$
Lake Panguipulli	57.9	$3.40 \pm 1.13^*$
Lotic populations:		
Botrolhue Stream	58.4	$3.40 \pm 0.96^*$
Peu-Peu Stream	65.3	$3.84 \pm 0.96^{**}$
<i>Unio tumidus</i> (2n = 38)	64.4	3.39

* $p > 0.05$.

** $p < 0.05$.

in this population, which may be associated with the occurrence of duplications of some DNA sequences, probably of constitutive heterochromatin. It should be possible to confirm the distribution and content of this chromatin within the karyotype of these populations by utilization of the C-banding technique (Martínez et al., 2001).

Based on the cytogenetic characteristics described for *D. ch. chilensis*, in conjunction with data already available in the literature for *Unio* (Barsienè, 1994), it is possible to suggest, at least in part, that chromosome morphology with submetacentric and metacentric tendencies is a highly conserved karyotype characteristic, probably ancestral at order level among the Unionoida. This chromosome morphology is also shared among species of other bivalve groups such as the Ostreidae, Mytilidae, and Pectinidae (Ladrón de Guevara et al., 1996; Palma-Rojas et al., 1997; Méndez et al., 2001).

Karyotype findings of this study do not support the proposal that the differences described by Parada & Peredo (1994) between river and lake populations of *D. ch. chilensis* may result from genetic differentiation among the populations. Therefore, on karyotype alone, there are not two categories of populations, lentic and lotic. In addition, the fact that the samples come from different hydrographic systems and drainages adds another uncontrolled variable, in that the discontinuity between two drainages itself implies some degree of isolation and reproductive discontinuity among the studied populations.

The cytogenetic information obtained in this study forms an initial contribution to the knowledge of cytogenetics characteristics in Chilean populations of *D. ch. chilensis*. This cytogenetic knowledge, together with mo-

phological, reproductive, and ecological contributions and the incorporation of molecular marker analysis, will facilitate a better understanding in the future of the evolutionary and taxonomic relationships between different populations of *Diplodon chilensis chilensis* and with other species of this genus and of other genera of the Hyriidae.

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